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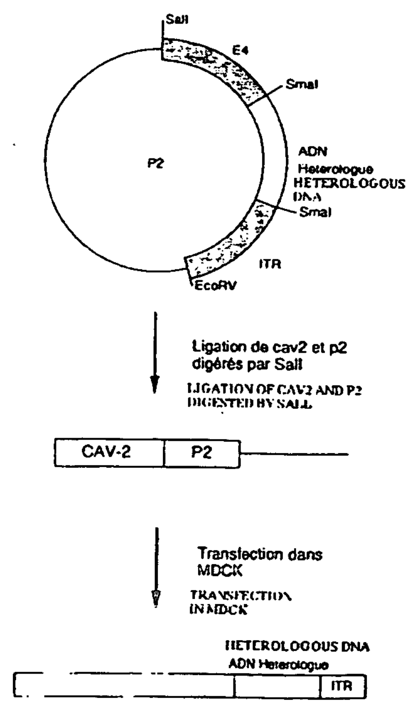
(57) Abstract

Use of a recombinant adenovirus of animal origin containing a heterologuous DNA sequence for the preparation of a pharmaceutical composition for use in the therapeutic and/or surgical treatment of the human body.

(57) Abrégé

La présente invention réside dans l'utilisation d'un adénovirus recombinant d'origine animale contenant une séquence d'ADN hétérologue pour la préparation d'une composition pharmaceutique destinée au traitement thérapeutique et/ou chirurgical du corps humain.



ADENOVIRAL VECTORS OF ANIMAL ORIGIN AND USE IN GENE THERAPY

The present invention relates to new viral vectors, to their preparation and to their use in gene therapy. It also relates to pharmaceutical compositions containing the said viral vectors. More especially, the present invention relates to the use of recombinant adenoviruses of canine origin as vectors for gene therapy.

Gene therapy consists in correcting a deficiency or an abnormality (mutation, aberrant expression, and the like) by introducing genetic information into the cell or organ affected. This genetic information may be introduced either in vitro into a cell extracted from the organ, the modified cell then being reintroduced into the body, or directly in vivo into the appropriate tissue. In this second place, different techniques exist, including various techniques of transfection involving complexes of DNA and DEAE-dextran (Pagano et al., J.Virol. 1 (1967) 891), of DNA and nuclear proteins (Kaneda et al., Science 243 (1989) 375) and of DNA and lipids (Felgner et al., PNAS 84 (1987) 7413), the use of liposomes (Fraley et al., J.Biol.Chem. 255 (1980) 10431), and the like. More recently, the use of viruses as vectors for gene transfer has been seen to be a promising alternative to these physical transfection techniques. In this connection, different viruses have been tested

for their capacity to infect certain cell populations. This applies especially to retroviruses (RSV, HMS, MMS, and the like), the HSV virus, adeno-associated viruses and adenoviruses.

Among these viruses, the adenoviruses display certain properties which are advantageous for use in gene therapy. In particular, they have a fairly broad host range, are capable of infecting resting cells and do not integrate in the genome of the infected cell. For these reasons, adenoviruses have already been used for in vivo gene transfer. To this end, different vectors derived from adenoviruses have been prepared, incorporating different genes (β-gal, OTC, α-1AT, cytokines, and the like). All the vectors derived from the adenoviruses described in the prior art for the purposes of use in gene therapy in humans have hitherto been prepared from adenoviruses of human origin. These appeared, in effect, to be the most suitable for such a use. To limit the risks of multiplication and the formation of infectious particles in vivo, the adenoviruses used are generally modified so as to render them incapable of replication in the infected cell. Thus, the constructions described in the prior art are adenoviruses from which the E1 (E1a and/or E1b) and, where appropriate, E3 regions have been deleted, at the site of which regions sequences of interest are inserted (Levrero et al., Gene 101 (1991) 195; Gosh-Choudhury et al., Gene 50 (1986) 161). However, in

addition to this necessary modification step, the vectors described in the prior art retain other drawbacks which limit their exploitation in gene therapy, and in particular risks of recombination with wild-type adenoviruses. The present invention provides an advantageous solution to this problem.

The present invention consists, in effect, in using recombinant adenoviruses of canine origin for gene therapy in humans. The present invention is the outcome of the demonstration that adenoviruses of canine origin are capable of infecting human cells very effectively. The invention is also based on the demonstration that adenoviruses of canine origin are incapable of propagating in the human cells in which they have been tested. Lastly, the invention is based on the surprising discovery that adenoviruses of canine origin are in no way trans-complemented by adenoviruses of human origin, thereby eliminating all risk of recombination and propagation in vivo in the presence of a human adenovirus, capable of leading to the formation of an infectious particle. The vectors of the invention are hence especially advantageous since the risks inherent in the use of viruses as vectors in gene therapy, such as pathogenicity, transmission, replication, recombination, and the like, are greatly reduced or even abolished.

The present invention thus provides viral vectors which are especially suitable for the transfer

and/or expression of desired DNA sequences in humans.

A first subject of the invention hence provides a method of therapeutic and/or surgical treatment of the human body comprising administering a recombinant adenovirus of canine origin containing a heterologous DNA sequence. The invention also relates to a recombinant adenovirus of canine origin.

In the context of the invention adenoviruses of canine origin are used, and in particular all strains of CAV-2 adenoviruses [strain manhattan or A26/61 (ATCC VR-800), for example]. Thus in the method of treatment of the invention the adenovirus may be a strain of CAV-2 adenovirus. Canine adenoviruses have formed the subject of many structural studies. Thus, complete restriction maps of CAV-1 and CAV-2 adenoviruses have been described in the prior art (Spibey et al., J. Gen. Virol. 70 (1989) 165), and the E1a and E3 genes as well as the ITR sequences have been cloned and sequenced (see, in particular, Spibey et al., Virus Res. 14 (1989) 241; Linné, Virus Res. 23 (1992) 119, WO 91/11525). Moreover, canine adenoviruses have already been used for the preparation of vaccines intended for immunizing dogs against rabies, parvoviruses, and the like (WO 91/11525). However, hitherto, the possibility of using these adenoviruses for gene therapy in humans has never been suggested in the prior art. Furthermore, the advantages

of such a use had never been weighed up.

The adenoviruses used in the context of the invention should preferably be defective, that is to say incapable of propagating autonomously in the body in which they are administered. As mentioned above, the Applicant has shown that adenoviruses of canine origin are capable of infecting human cells but not of propagating therein. In this sense, they are hence naturally defective in humans and, in contrast to the use of human adenoviruses, do not require genetic modification in this connection. However, the defective character of these adenoviruses may be amplified by genetic modifications of the genome, and in particular by modification of the sequences needed for replication of the said virus in cells. These regions may be either removed (wholly or partially), or rendered non-functional, or modified by insertion of other sequences, and in particular of the heterologous DNA sequence. Thus in the method of treatment of the invention the genome of the adenovirus lacks sequences needed for replication. The genome may lack the E1A and E1B regions.

The genome of the adenovirus of the invention discussed below may also lack at least the sequences needed for replication.

The sequences needed of replication are generally localized close to the ends of the genome.

Thus, in the case of CAV-2, the Ela region has been identified, cloned and sequenced (Spibey et al., Virus Res. 14 (1989) 241). It is located on the 2-kb fragment at the left-hand end of the adenovirus genome.

Moreover, other genetic modifications may be performed on the adenoviruses used in the method of treatment of the invention, in particular in order to avoid the production of viral proteins which are undesirable in humans, to permit the insertion of large heterologous DNA sequences and/or to insert particular regions of the genome of another animal or human adenovirus (example: E3 gene).

For the purpose of the present invention, the term "heterologous DNA sequence" denotes any DNA sequence introduced into the virus, the transfer and/or expression of which in humans is desired.

In particular, the heterologous DNA sequence can contain one or more therapeutic genes and/or one or more genes coding for antigenic peptides.

In a particular embodiment of the method of treatment of the invention the adenovirus transfers a therapeutic gene to the human body. The therapeutic gene which may be transferred in this way is any gene, the transcription and, where appropriate, translation of which in the target cell generates a product(s) having a therapeutic effect.

It can be, in particular, a gene coding for a therapeutic proteinaceous product. The proteinaceous product thus encoded can be a protein, a peptide, an amino acid, and the like. This proteinaceous product can be homologous with respect to the target cell (that is to say a product which is normally expressed in the target cell when the latter does not display any pathology). In this case, the expression of a protein makes it possible, for example, to compensate for an insufficient expression in the cell or for the expression of a protein that is inactive or poorly active as a result of a modification, or alternatively to overexpress the said protein. The therapeutic gene can also code for a mutant of a cellular protein, having enhanced stability, modified activity, and the like. The proteinaceous product can also be heterologous with respect to the target cell. In this case, an expressed protein can, for example, supplement or supply an activity which is deficient in the cell, enabling it to combat a pathology.

Among products which are therapeutic for the purpose of the present invention, there may be mentioned, more especially, enzymes, blood derivatives, hormones, lymphokines, namely interleukins, interferons, and the like (FR 92/03120), growth factors, neurotransmitters or their precursors or synthetic enzymes, trophic factors, namely BDNF, CNTF, NGF, IGF, GMF, aFGF, bFGF, NT3, NT5,

and the like; apolipoproteins, namely ApoAI, ApoAIV, ApoE, and the like (FR 93/05125), dystrophin or a minidystrophin (FR 91/11947), tumour-suppressing genes, namely p53, Rb, Rap1A, DCC, k-rev, and the like (FR 93/04745) and genes coding for factors involved in coagulation, namely factors VII, VIII, IX, and the like.

The invention also provides a recombinant adenovirus of canine origin, containing at least one inserted gene coding for a therapeutic protein product chosen from enzymes, blood derivatives, hormones, lymphokines, growth factors, neurotransmitters or their precursors or synthetic enzymes, trophic factors, apolipoproteins, dystrophin or a minidystrophin, tumour-suppressors and factors involved in coagulation. The adenovirus of the invention may be a strain of CAV-2 adenovirus.

The therapeutic gene of the adenovirus of the method of the treatment of the invention can also be a gene or sequence, expression of which in the target cell enables the expression of cellular genes or the transcription of cellular mRNA to be controlled.

As mentioned above, the heterologous DNA sequence can also contain one or more genes coding for an antigenic peptide capable of generating an immune response in humans. In this particular embodiment, the invention hence makes it possible to produce vaccines

enabling humans to be immunized, in particular against microorganisms or viruses. Such antigenic peptides can be, in particular specific to the Epstein-Barr virus, the hepatitis B virus (EP 185,573) or the pseudorabies virus, or alternatively tumour-specific (EP 259,212).

Generally, the heterologous DNA sequence also comprises sequences permitting expression of the therapeutic gene and/or of the gene coding for the antigenic peptide in the infected cell. Such sequences can be the ones which are naturally responsible for expression of the gene in question when these sequences are capable of functioning in the infected cell. They can also be sequences of different origin (responsible for the expression of other proteins, or even synthetic). In particular, they can be promoter sequences of eukaryotic or viral genes. For example, they can be promoter sequences originating from the genome of the cell which it is desired to infect. Similarly, they can be promoter sequences originating from the genome of a virus, including the adenovirus used. The promoters of the E1A, MLP, CMV, RSV, and the like, genes may be mentioned, for example, in this connection. In addition, these expression sequences may be modified by the addition of activating, regulating, and the like, sequences. Moreover, when the inserted gene does not contain expression sequences, it may be inserted into the

genome of the defective virus downstream of such a sequence.

The adenovirus of the invention may comprise promoter sequences permitting expression of the inserted therapeutic gene or genes.

Moreover, the heterologous DNA sequence of the adenovirus of the method of treatment of the invention can also contain, especially upstream of the therapeutic gene, a signal sequence directing the synthesized therapeutic product into the pathways of secretion of the target cell. This signal sequence can be the natural signal sequence of the therapeutic product, but it can also be any other functional signal sequence or an artificial signal sequence. The adenovirus of the invention may comprise signal sequences enabling a secretion of the expression product of the therapeutic gene to be induced.

The defective adenovirus according to the invention may be prepared by any technique known to a person skilled in the art. In particular, they may be prepared according to the protocol described in application WO 91/11525. The traditional preparation technique is based on homologous recombination between a canine adenovirus and a plasmid carrying, inter

alia, the heterologous DNA sequence which it is desired to insert. Homologous recombination takes place after cotransfection of the said adenovirus and plasmid into a suitable cell line. The cell line used should preferably be transformable by the said elements and, in the case where a modified adenovirus of canine origin is used, the cell line can, if necessary, contain sequences capable of complementing the portion of the defective adenovirus genome, preferably in integrated form in order to avoid risks of recombination. As an example of a line, the GHK greyhound kidney cell line (Flow laboratories) or the MDCK cell line may be mentioned. The conditions of culture of cells and of preparation of the viruses or of the viral DNA have also been described in the literature (see, in particular, Macatney et al., Science 44 (1988) 9; Fowlkes et al., J. Mol. Biol. 132 (1979) 163).

Thereafter, the vectors which have multiplied are recovered and purified according to the traditional techniques of molecular biology.

Another subject of the present invention relates to a recombinant adenovirus of canine origin containing a heterologous DNA sequence comprising at least one therapeutic gene as defined above.

The subject of the invention is also a pharmaceutical composition comprising one or more adenoviruses of the invention. The

pharmaceutical compositions of the invention may be formulated with a view to topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous, intraocular, and the like, administration.

Preferably, the pharmaceutical composition contains vehicles which are pharmaceutically acceptable for an injectable formulation. These can be, in particular, sterile, isotonic saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride, and the like, or mixtures of such salts), or dry, in particular lyophilized, compositions which, on adding sterilized water or physiological saline, as the case may be, enable injectable solutions to be formed.

The doses of virus used for the injection may be adapted in accordance with different parameters, and in particular in accordance with the mode of administration used, the pathology in question, the gene to be expressed or the desired period of treatment. Generally speaking, the recombinant adenoviruses according to the invention are formulated and administered in the form of doses of between 10^4 and 10^{14} pfu/ml, and preferably 10^6 to 10^{10} pfu/ml. The term pfu (plaque forming unit) corresponds to the infectious power of a solution of virus, and is determined by infecting a suitable cell culture and measuring, generally after 5 days, the number of plaques of infected cells. The techniques of determination of the

pfu titre of a viral solution are well documented in the literature.

Depending on the heterologous DNA sequence inserted, the adenoviruses of the invention may be used for the treatment or prevention of a large number of pathologies, including genetic diseases (dystrophy, cystic fibrosis, and the like), neurodegenerative diseases (Alzheimer's, Parkinson's, ALS, and the like), cancers, pathologies associated with disorders of coagulation or with dislipoproteinaemias, pathologies associated with viral infections (hepatitis, AIDS, and the like), and the like.

The present invention will be described more completely by means of the examples which follow, which should be considered to be illustrative and non-limiting.

Legends to the figures

Figure 1: Restriction map of the CAV2 adenovirus strain Manhattan (according to Spibey et al., cited above).

Figure 2: Map of plasmids p1, p2 (Figure 2a) and p3 (Figure 2b).

Figure 3: Strategy for the construction of a recombinant canine adenovirus containing a heterologous DNA sequence.

General techniques of molecular biology

The methods traditionally used in molecular

biology, such as preparative extractions of plasmid DNA, centrifugation of plasmid DNA in a caesium chloride gradient, agarose or acrylamide gel electrophoresis, purification of DNA fragments by electroelution, phenol or phenol-chloroform extraction of proteins, ethanol or isopropanol precipitation of DNA in a saline medium, transformation in Escherichia coli, and the like, are well known to a person skilled in the art and are amply described in the literature [Maniatis T. et al., "Molecular Cloning, a Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982; Ausubel F.M. et al. (eds), "Current Protocols in Molecular Biology", John Wiley & Sons, New York, 1987].

Plasmids of the pBR322 and pUC type and phages of the M13 series are of commercial origin (Bethesda Research Laboratories).

To carry out ligation, the DNA fragments may be separated according to their size by agarose or acrylamide gel electrophoresis, extracted with phenol or with a phenol-chloroform mixture, precipitated with ethanol and then incubated in the presence of phage T4 DNA ligase (Biolabs) according to the supplier's recommendations.

The filling in of 5' protruding ends may be performed with the Klenow fragment of E.coli DNA polymerase I (Biolabs) according to the supplier's specifications. The destruction of 3' protruding ends

is performed in the presence of phage T4 DNA polymerase (Biolabs) used according to the manufacturer's recommendations. The destruction of 5' protruding ends is performed by a controlled treatment with S1 nuclease.

Mutagenesis directed in vitro by synthetic oligodeoxynucleotides may be performed according to the method according to Taylor et al. [Nucleic Acids Res. 13 (1985) 8749-8764] using the kit distributed by Amersham.

The enzymatic amplification of DNA fragments by the so-called PCR [Polymerase-catalysed Chain Reaction, Saiki R.K. et al., Science 230 (1985) 1350-1354; Mullis K.B. and Faloona F.A., Meth. Enzym. 155 (1987) 335-350] technique may be performed using a DNA thermal cycler (Perkin Elmer Cetus) according to the manufacturer's specifications.

The verification of the nucleotide sequences may be performed by the method developed by Sanger et al. [Proc. Natl. Acad. Sci. USA, 74 (1977) 5463-5467] using the kit distributed by Amersham.

Examples

E1. Infection of human cells with adenoviruses of canine origin.

This example demonstrates the capacity of adenoviruses of animal (canine) origin to infect human cells.

E1.1. Cell lines used

In this example, the following cell lines were used:

- Human embryonic kidney line 293 (Graham et al., J. Gen. Virol. 36 (1977) 59). This line contains, in particular, integrated in its genome, the left-hand portion of the Ad5 human adenovirus genome(12%).

- KB human cell line: Originating from a human epidermal carcinoma, this line is available in the ATCC (ref. CCL17), together with the conditions enabling it to be cultured.

- Hela human cell line: Originating from a human epithelial carcinoma, this line is available in the ATCC (ref. CCL2), together with the conditions enabling it to be cultured.

- MDCK canine cell line: The conditions of culture of MDCK cells have been described, in particular, by Macatney et al., Science 44 (1988) 9.

E1.2. Infection

Cells of the cell lines mentioned above are infected with CAV2 virus (strain Manhattan). For this purpose, the cells (approximately 10^7/dish) were incubated for 1 hour at 37°C in the presence of 10 pfu/cell of virus. 5 ml of culture medium were then added and culturing was continued at 37°C for approximately 48 hours. At this point, the DNA present in episomal form in the infected cells was analysed: the results obtained show that all the cell lines

possess CAV2 DNA in their nuclei, thereby demonstrating that they can be infected by canine adenoviruses.

E2. Absence of propagation of adenoviruses of canine origin in human cells.

This example demonstrates that canine adenoviruses, although capable of infecting human cells, do not propagate therein.

After infection of cells according to Example 1, the quantity of CAV2 DNA was assayed over time according to the following protocol: the episomal DNA present in the cells was recovered according to the technique described by Hirt et al. (J. Virol. 45 (1983) 91), and the quantity of DNA was assayed by comparison with a standard series. The results obtained show that the quantity of viral DNA does not increase in KB and 293 cells, demonstrating a complete absence of replication of CAV2 in these cells. In MDCK and Hela cells, a slight increase in the quantity of CAV2 viral DNA is observed. However, assay of the formation of viral particles shows that no propagation of CAV2 takes place in 293, KB and Hela human cells, only in the MDCK canine line. Propagation was measured by harvesting the infected cells, releasing any viruses by freezing/thawing and infecting MDCK cells with the supernatant thereby obtained, under the conditions described above. After 48 hours of culture, the absence of viral DNA in MDCK cells infected in this manner

demonstrates that no viral propagation has taken place in the human cells.

These results show clearly that canine adenoviruses are incapable of propagating in human cells.

E3. Demonstration of the absence of trans-complementation of canine adenoviruses by human adenoviruses.

This example shows that the absence of propagation of canine adenoviruses in human cells is not trans-complemented by the presence of human adenoviruses.

Cells of the 293, KB and Hela human lines and of the MDCK canine line were coinfected with CAV2 adenovirus and Ad5 human adenovirus. The presence of viral (canine and human) DNA in the cells was demonstrated as in Example E1, and the quantity of DNA was assayed over time, together with propagation. The results obtained show that the quantity of CAV2 DNA does not increase over time in KB and 293 cells, thereby demonstrating that the presence of Ad5 human adenovirus does not induce replication of CAV2 in these cells by trans-complementation. The absence of viral DNA in the MDCK cells infected with the possible viruses originating from the KB, 293 and Hela cells likewise demonstrates that no propagation of the CAV2 adenovirus in the human cell lines has taken place,

even in the presence of human adenovirus.

E4. Construction of a CAV2 genomic DNA library.

A library of plasmids was constructed from restriction fragments of the CAV2 adenovirus genome. This library was obtained by digestion of CAV2 with the enzymes SmaI and PstI and cloning of the SmaI fragments, namely A, B, C, D, E, F, I and J, and PstI fragments, namely A, B, C, D, E, F, G and H (Figure 1) into the vector pGem3Zf+ (Promega). The plasmid carrying the SmaI fragment C was then cotransfected into MDCK cells with plasmid pUC4KIXX (Pharmacia) carrying the neomycin resistance gene, to establish an MDCK line constitutively expressing the E1A and E1B genes of CAV2. This line permits the construction of recombinant viruses from which these regions have been deleted (see E5.2.).

E5. Construction of recombinant canine adenoviruses carrying the interleukin-2 gene under the control of the MLP promoter of Ad2.

Two strategies for the construction of recombinant canine adenoviruses carrying the interleukin-2 gene under the control of the MLP promoter of human Ad2 were devised.

E5.1. The first consists in inserting the desired heterologous DNA sequence (MLP promoter - interleukin-2 gene) between the E4 region and the right-hand ITR of the entire CAV2 genome at the SmaI restriction site. The construction of such a recombinant is performed either by ligation, or by in vivo recombination between a plasmid carrying the desired heterologous DNA sequence and the CAV2 genome. The plasmids used for obtaining the recombinant adenoviruses carrying the interleukin-2 gene under the control of the MLP promoter are constructed in the following manner (Figure 2):

- a first plasmid, designated p1, is obtained by cloning the SalI fragment B of CAV2 (fig 1), containing, in particular, the right-hand ITR, a SmaI site and the E4 gene, into plasmid pGem3Zf+ (Promega); the SmaI site is unique on p1;

- the heterologous DNA sequence (MLP promoter - interleukin-2 gene) is introduced at the SmaI site of plasmid p1 to generate plasmid p2; and

- the PstI-SalI fragment contained in the PstI fragment D of the genomic library and carrying a portion of the E3 gene is then cloned at the corresponding site in p2 to generate plasmid p3 (Figure 2).

The plasmids thereby obtained are used to prepare the recombinant adenoviruses according to the following two protocols (see Figure 3):

a) in vitro ligation of plasmid p2 digested with SalI to the SalI fragment A of CAV2, and transfection of the ligation product into MDCK cells (Figure 3a),

b) Recombination between plasmid p3 and the SalI fragment A of CAV2 after cotransfection into MDCK cells (Figure 3b). The recombinant adenoviruses obtained are then isolated and amplified according to techniques known to a person skilled in the art.

These manipulations permit the construction of recombinant canine adenoviruses carrying the interleukin-2 gene, which are usable for the transfer of this therapeutic gene in man (Figure 3).

E5.2. The second strategy devised is based on the use of MDCK cell line constitutively expressing the E1A and E1B genes of CAV2 (see Example E4). This line permits, in effect, trans-complementation of canine adenoviruses from which these regions have been deleted, and thus the construction of recombinant viruses in which the heterologous DNA sequence is substituted for the E1 region. For this purpose, a plasmid containing the left-hand end (ITR sequence and encapsidation sequence) of the CAV2 genome and the heterologous DNA sequence is constructed. This plasmid is cotransfected into the MDCK cells described above, in the presence of the genome of a CAV2 adenovirus from which its own left-hand end has been deleted. The recombinant canine adenoviruses produced are recovered,

where appropriate amplified and stored for the purpose of using them in humans.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Method of therapeutic and/or surgical treatment of the human body comprising administering a recombinant adenovirus of canine origin containing a heterologous DNA sequence.

2. Method according to claim 1, wherein the adenovirus transfers a therapeutic gene to the human body.

3. Method according to claim 2, characterised in that the therapeutic gene is a gene coding for a therapeutic proteinaceous product.

4. Method according to any one of claims 1 to 3, characterised in that the adenovirus is a strain of CAV-2 adenovirus.

5. Method according to any one of claims 1 to 4, characterised in that the genome of the adenovirus lacks the sequences needed for replication.

6. Method according to claim 5, characterised in that the genome lacks the E1A and E1B regions.

7. Recombinant adenovirus of canine orgin, containing at least one inserted gene coding for a therapeutic protein product chosen from enzymes, blood derivatives, hormones, lymphokines, growth factors, neurotransmitters or their precursors or synthetic enzymes, trophic factors, apolipoproteins, dystrophin or a minidystrophin, tumour-suppressors and factors involved in coagulation.

8. Adenovirus according to claim 7, characterised in that it also comprises promoter sequences permitting expression of the inserted therapeutic gene or genes.

9. Adenovirus according to claim 7 or 8, characterised in that it also comprises signal sequences enabling a secretion of the expression product of the therapeutic gene to be induced.

10. Adenovirus according to any one of claims 7 to 9, characterised in that the adenovirus is a CAV-2 adenovirus.

11. Adenovirus according to one of claims 7 to 10, characterised in that its genome lacks at least the sequences needed for replication.

12. Adenovirus according to claim 11, characterised in that it comprises regions of another animal or human adenovirus.

13. Pharmaceutical composition comprising one or more adenoviruses according to any one of claims 7 to 12.

14. Use of a recombinant adenovirus of canine origin containing a heterologous DNA sequence for the preparation of a pharmaceutical composition intended for the therapeutic and/or surgical treatment of the human body.

15. Use according to claim 14, for the preparation of a pharmaceutical composition intended for the transfer of a therapeutic gene to the human body.

16. Use according to claim 15, characterised in that the therapeutic gene is a gene coding for a therapeutic proteinaceous product.

17. Use according to claim 16, characterised in that the adenovirus is a strain of CAV-2 adenovirus.

18. Use according to one of claims 14 to 17, characterised in

that the genome of the adenovirus lacks the sequences needed for replication.

19. Use according to claim 18, characterised in that the genome lacks the E1A and E1B regions.

Dated this 3rd day of August 1998.

Rhone-Poulenc Rorer S.A.

By its Patent Attorneys

Davies Collison Cave

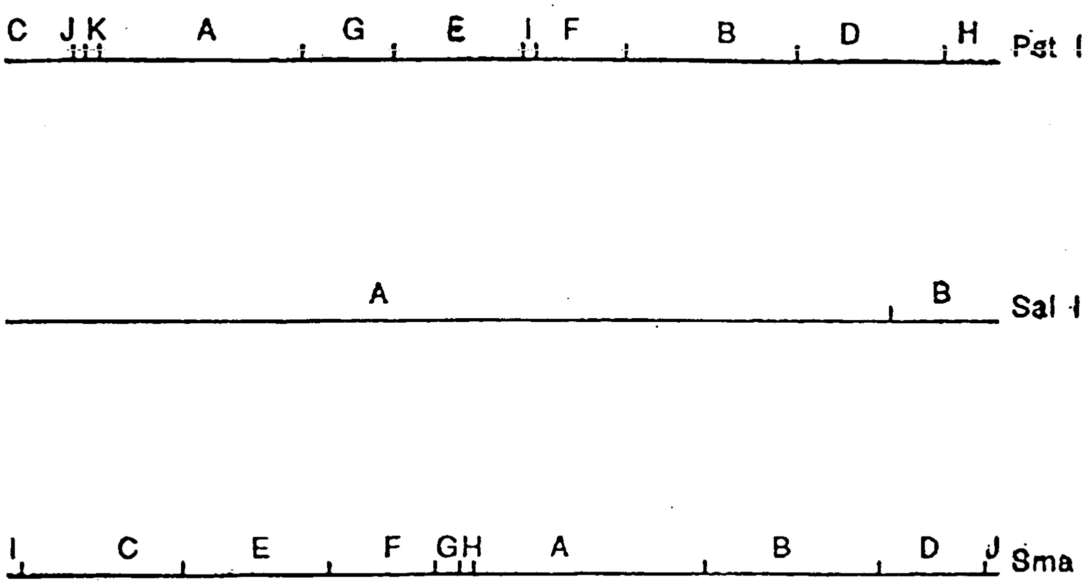


Figure 1

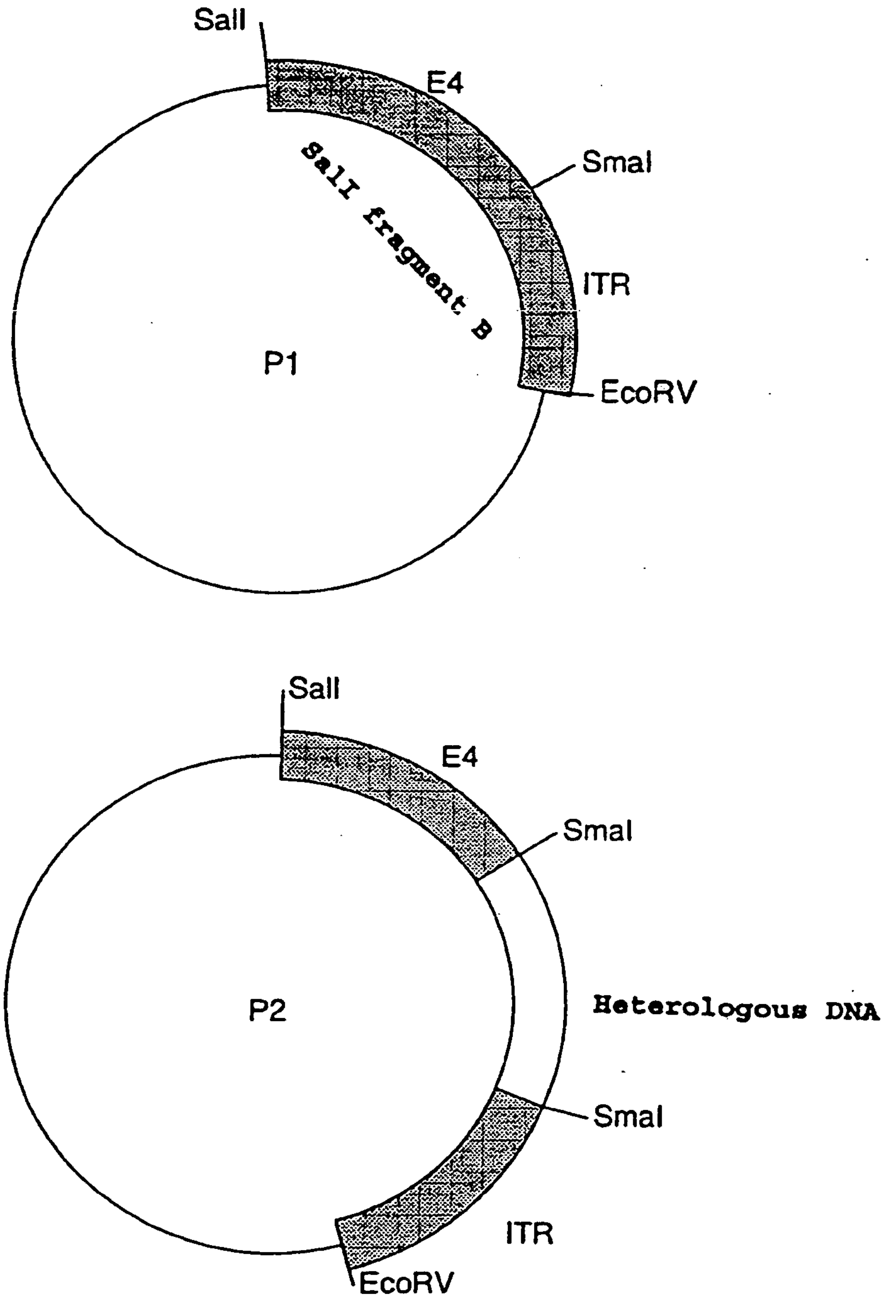

SalI
E4
SmaI
SalI fragment B
ITR
P1
EcoRV
SalI
E4
SmaI
P2
Heterologous DNA
SmaI
ITR
EcoRV

Figure 2a

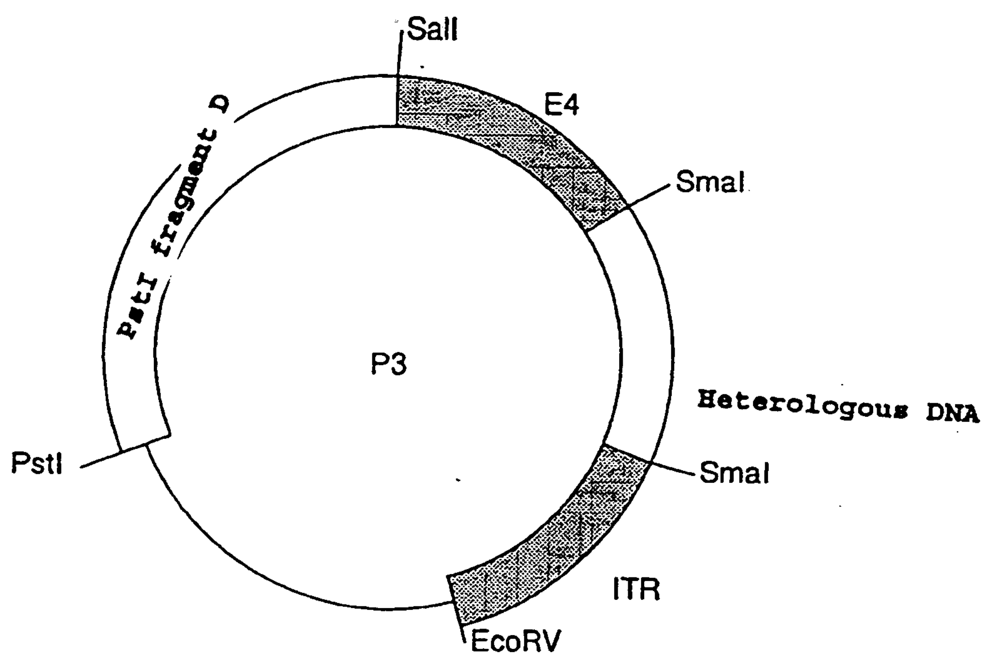

SalI
E4
SmaI
PstI fragment D
P3
Heterologous DNA
SmaI
PstI
ITR
EcoRV

Figure 2b

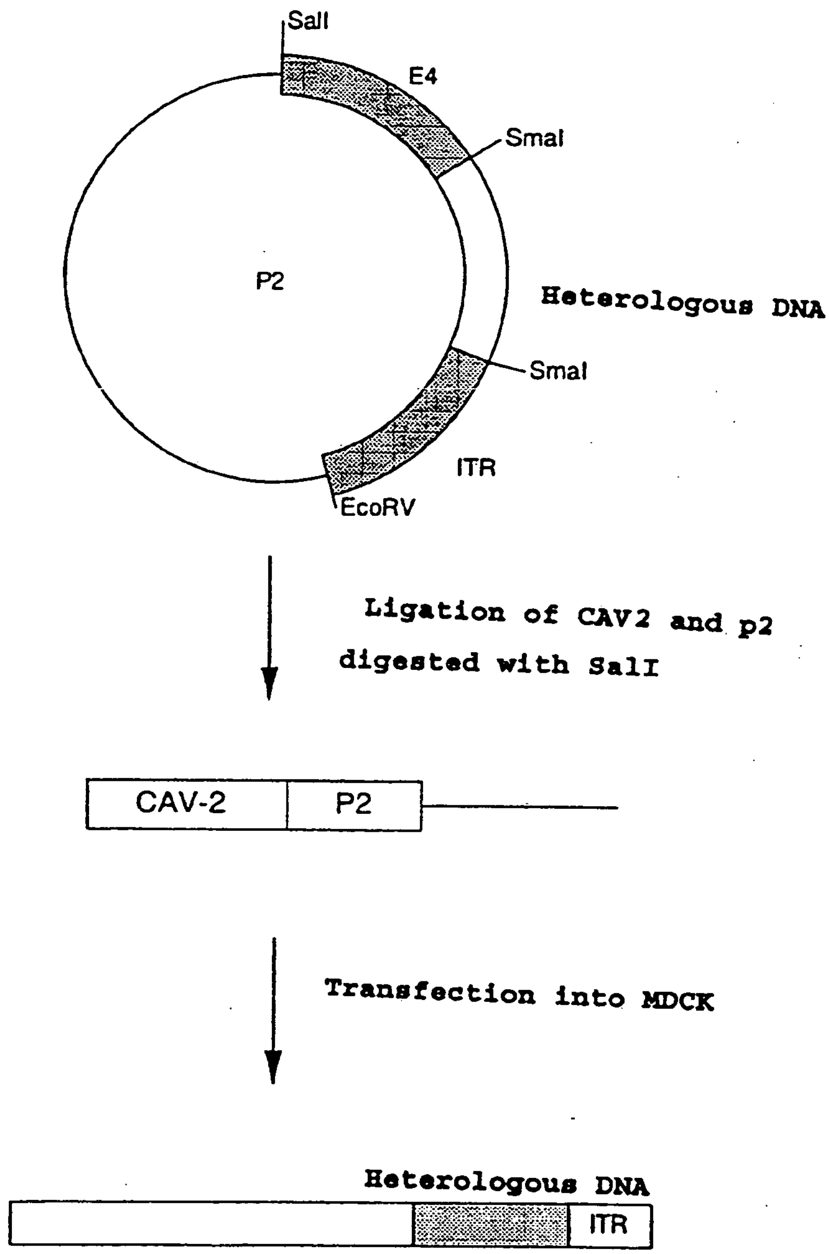

SalI
E4
SmaI
P2
Heterologous DNA
SmaI
ITR
EcoRV
Ligation of CAV2 and p2
digested with SalI
CAV-2
P2
Transfection into MDCK
Heterologous DNA
ITR

Figure 3a

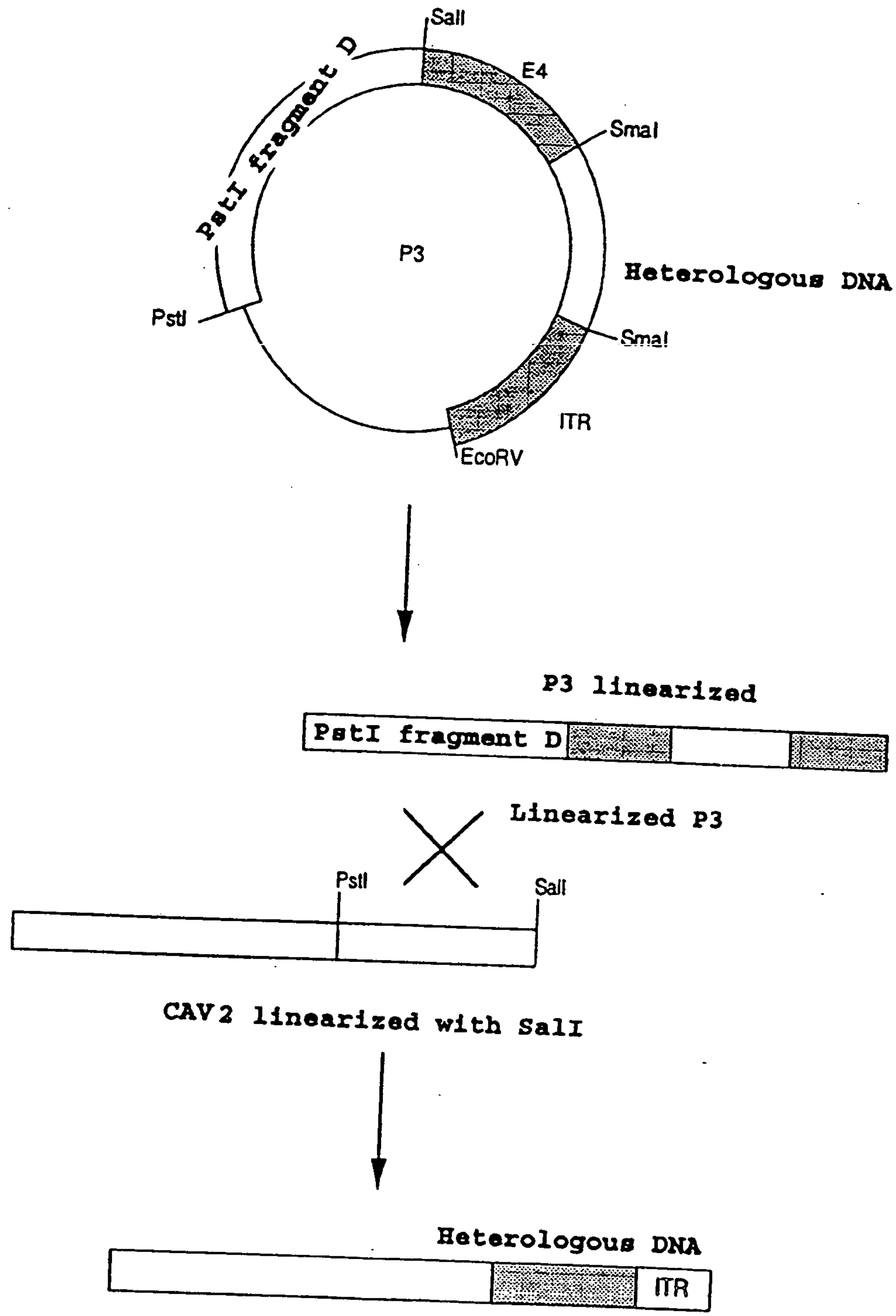

SalI
E4
SmaI
PstI fragment D
P3
Heterologous DNA
PstI
SmaI
ITR
EcoRV
P3 linearized
PstI fragment D
Linearized P3
PstI
SalI
CAV2 linearized with SalI
Heterologous DNA
ITR

Figure 3b

IN THE MATTER OF an Australian Application corresponding to PCT Application PCT/FR94/00531

I, Abraham SMITH DipIng DipDoc, c/o Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England, do solemnly and sincerely declare that I am conversant with the English and French languages and am a competent translator thereof, and that to the best of my knowledge and belief the following is a true and correct translation of the replacement sheets of the PCT Application filed under No. PCT/FR94/00531.

Date: 25 October 1995

Abra Smith

A. SMITH

For and on behalf of RWS Translations Ltd.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR 94/00531

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/86 C12N15/26 C07K13/00 A61K48/00 C12N7/01 A61K39/235

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 06223 (CNRS) 1 April 1993 see the whole document ---	1
A	WO,A,91 11525 (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW) 8 August 1991 see the whole document ---	1
P,A	WO,A,93 19191 (CNRS; INSTITUT GUSTAVE ROUSSY) 30 September 1993 see the whole document -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 1994	- 4. 08. 94

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/FR 94/00531

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		N/A AU-B-	3757093	21-10-93 672195
		N/A CA-A-	2102302	17-09-93
		EP-A-	0593755	27-04-94